

# General Biochemistry

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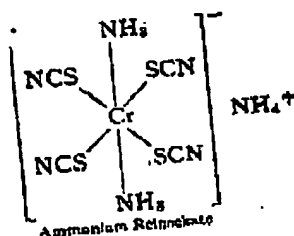
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## Structural Units of Proteins

of chemical substances some-  
since they were first studied  
during the period 1900-  
soluble salt with proline  
late may therefore be obtained

s not react with nitrous acid to  
so derivative is formed which is  
tion has been used for the de-  
protein hydrolysates.<sup>10</sup>  
ine-2-carboxylic acid) was first  
fischer in 1902. This amino acid  
proteins, but is found in relatively  
cent). Its isolation from protein  
can found that, after removal of  
proline could be precipitated as a  
inner complex, termed ammonium



## acid Chemistry to Protein Analysis

discussion, reference has been made to  
the characteristic of the side chains of  
the nitroprusside test (sulfhydryl  
guanidino group), the Pauly reaction  
teic reaction (phenols), and the Mil-  
these reactions are also given by pro-  
amino acids on hydrolysis.<sup>11, 12</sup> This  
in the unhydrolyzed protein, the side  
c not so substituted as to make them  
ion with these reagents. These color

J. Biol. Chem., 184, 607 (1950).  
rotein Chem., 3, 169 (1947).  
Contrat. Chem. Revs., 41, 151 (1947).

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### Applications to Protein Analysis

reactions have long been used in qualitative tests for proteins in natural materials. It is of interest that protein-bound tryptophan does not respond to the Hopkins-Cole reaction.

Some of these reactions have also been used to good advantage in attempts to study proteins in intact cells. Of especial interest in such cytochemical studies is the use of ultraviolet absorption spectroscopy for the determination of protein concentration in cells and tissues. Since the ultraviolet absorption of protein solutions is of considerable practical value in protein studies, a brief discussion of the principles involved is desirable.

It will be recalled that the absorption of monochromatic light by a solution may be described by Beer's law, which states that the absorbancy, formerly termed optical density ( $d$ ), of the solution is given by the expression  $\log(I_0/I)$ , where  $I_0$  is the intensity of the incident light, and  $I$  is the intensity of the emergent light. The absorption coefficient (or extinction coefficient)  $E$  is defined as the optical density for a light path ( $l$ ) of 1 cm; thus,  $E = d/l$ . If one wishes to express the light absorption in terms of the molar concentration ( $c$ ) of the absorbing material in solution, the molar absorption coefficient ( $E_{mol}$ ) is given by the equation  $E_{mol} = E/c$ . If the value of  $E$  (or  $E_{mol}$ ) at various wave lengths of light is plotted as the ordinate against the wave length as the abscissa, a curve results which gives the absorption spectrum of the absorbing material in the solution. Most modern instruments designed for this purpose permit the accurate estimation of the optical density of a solution at narrowly spaced intervals from about 200  $m\mu$  (2000 Å) to about 650  $m\mu$  (6500 Å). [1  $m\mu$  = 10 Å (angstrom units) =  $10^{-7}$  cm.] Visible light is composed of light rays having wave lengths from about 400  $m\mu$  (violet) to about 650  $m\mu$  (red), and the region below 400  $m\mu$  is termed the ultraviolet region of the spectrum.

Most organic substances absorb light of wave lengths below 250  $m\mu$ ; the absorption of light of longer wave lengths is usually associated with the presence, in the molecule, of unsaturated linkages. In general, an increase in the number of unsaturated linkages and their presence in conjugated systems contribute to light absorption at longer wave lengths.

Of the widely distributed protein amino acids, only phenylalanine, tyrosine, and tryptophan exhibit extensive light absorption at wave lengths longer than 250  $m\mu$ ; this may be attributed to their aromatic nature. The absorption spectra of these amino acids are given in Fig. 1, and it will be seen that phenylalanine exhibits maximal absorption at about 250  $m\mu$ , whereas tyrosine and tryptophan have their absorption maxima at about 275  $m\mu$  and 280  $m\mu$ , respectively. If a protein contains one or more of these amino acids, therefore, an aqueous solution of the protein

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### Amino Acids as Structural Units of Proteins

will absorb light in the region 260 to 290  $m\mu$ , and this property may be used to measure its concentration. Since the relative proportions and absolute content of the three amino acids vary widely from one protein

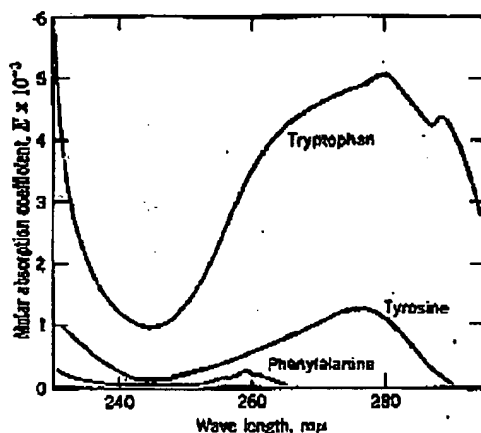


FIG. 1. Ultraviolet absorption spectra of aromatic amino acids derived from proteins (pH 8).

to another, each protein will, in general, exhibit a different value for the wave length of maximal absorption and the extinction coefficient. Thus, a one per cent solution of human serum albumin exhibits maximal absorption at 280  $m\mu$ , where the value of  $E$  is 5.32; on the other hand, a one per cent solution of beef insulin absorbs maximally at 277  $m\mu$ , and the value for  $E$  is 11.3. Clearly, the use of such values is justified only when one is dealing with solutions in which no other substances absorb light appreciably near 280  $m\mu$ .

### Optical Activity of Amino Acids

Attention must now be given to the property of amino acids, when in solution that enables them to rotate the plane of polarized light. This property is termed optical activity. A brief outline of the basic concepts involved is given in what follows; a more complete discussion may be found in the treatise by Lowry.<sup>12</sup>

<sup>12</sup> T. M. Lowry, *Optical Rotatory Power*, Longmans, Green and Co., London, 1935.

### Optical Activity of Amino

In 1669 it was found that of Iceland spar (a transparent crystal) results. This was experimentally shown that when a ray of light is passed through a crystal, two rays are formed. One of these, (bent) in accord with Snell's law, is called the "ordinary" ray, was refracted at the angle that the incident ray makes with the normal to the surface of the crystal. The other ray, called the "extraordinary" ray, was refracted at a different angle, the phenomenon of "double refraction".

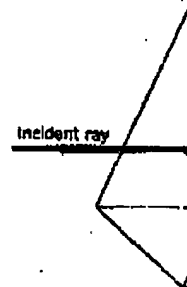


FIG. 2. Double refraction

of the two rays, so that one of them is perpendicular to the plane of vibration of the light. In 1828 Nicol described the method of producing polarized light by double refraction. Iceland spar cut from a single crystal and cemented together with Canada balsam through this prism at the point where the extraordinary ray is transmitted, the darkened lateral faces of the prism are allowed to fall upon each other. If the orientation of the second prism is rotated through 90 degrees, light will be absorbed in the second prism. If the light has been absorbed in the first prism, and if the second prism is rotated through 90 degrees, the light will be transmitted through both prisms. They are said to be in the same plane of vibration.

During the early part of the 19th century, the Biot found that quartz crystals rotate the plane of the polarized light.